Studies on the Binding of *Xenopus* Transcription Factor (TFIIIA) to the 5S RNA Gene by Scanning Transmission Electron Microscopy.

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ABSTRACT

Complexes of the *Xenopus laevis* somatic 5S gene-specific transcription factor, TFIIIA, and cloned somatic 5S DNA have been examined by scanning transmission electron microscopy (STEM). DNA contour length implies that the length of the DNA is compatible with form B DNA. Mass measurement of TFIIIA specifically bound to DNA as determined by the STEM, Mr=40,000 ± 4000 is in agreement with previous values obtained by gel migration. Preliminary measurement of the binding of TFIIIA to the 5S gene suggests that TFIIIA prefers to bind to the 3′ half of the internal control region.

INTRODUCTION

A somatic 5S specific transcription factor from *Xenopus laevis* designated TFIIIA has been shown to mediate specific transcription of 5S RNA (1) and considerable progress has been made toward an understanding of the role of TFIIIA in 5S gene transcription. TFIIIA has been shown to interact specifically with the intragenic control regions in the 5S ribosomal RNA genes of *Xenopus* (1, 2, 3, 4) and in addition contact points between TFIIIA and *Xenopus* 5S RNA gene within the control region have been studied (5). This interaction is an essential part necessary for accurate transcription initiation. Scanning transmission electron microscopy (STEM) should be able to contribute strongly to a detailed description of this interaction between TFIIIA and 5S RNA gene. STEM has been shown to be a valuable technique which allows one to examine protein structure and to simultaneously measure molecular weight (6, 7, 8). Moreover, because one can directly examine unstained protein, the technique is free from many of the usual

artifacts of sample preparation. In the present study, the position of TFIIIA relative to the control region of *Xenopus laevis* 5S DNA is studied and the mass of specifically bound TFIIIA determined using the STEM.

MATERIALS AND METHODS

Donated Materials: *Xenopus laevis* 5S DNA (Xls 5S DNA) fragments were a gift from Angela Kramer and were purified from cloned *X. laevis* somatic 5S DNA (Xlsl1) by the method described by Peterson et al. (9). TFIIIA was a gift from Angela Kramer. It was purified from immature *X. laevis* ovaries as described by Pelham and Brown (10).

Binding of TFIIIA to Xenopus laevis DNA: The binding of TFIIIA to DNA was carried out in binding buffer containing 10 mM Hepes (pH 7.5), 8% glycerol, 6 mM $MgCl_2$, 70 mM KCl. TFIIIA was diluted to 13.5 μg/ml in binding buffer and incubated at room temperature for 15 min. Diluted TFIIIA was added to 10 μl of DNA previously diluted to 10 μg/ml in binding buffer and incubated at room temperature for 10 min which is 8 : 1 molar ratio of TFIIIA to DNA.

STEM: Specimens were examined in the Brookhaven STEM Biotechnology Resource (6, 7, 11). Carbon film grids were prepared by picking up thin carbon film floated from rock salt onto the titanium grids which carry a "holey" film of carbon (12) the day of the experiment. The grid is discharged in pure N_2 at a pressure of 200 millitorr for 30 seconds, within a two hour period before use. A droplet of 3.5 μl containing TFIIIA and DNA were applied to the carbon film for 2 min. Specimens were fixed 30 seconds in 0.03% (v/v) glutaraldehyde. Tabacco mosaic virus (TMV) was added as a standard and washed five times with twice-distilled water. With just a thin layer of water remaining, the grids were frozen by immersion liquid N_2, freeze-dried by sublimation overnight in an oil-free vacuum system with the temperature held low enough that the vapor pressure does not exceed 10^{-7} Torr and introduced into the microscope vacuum without being exposed to a pressure greater than 10^{-4} Torr. The grids were not stained or shadowed.

Mass Analysis: The images obtained from the STEM were recorded in dig-

ital form as intensity of electron scattering as a function of position (512 × 512 sampling positions per field; a field is 0.5 μm × 0.5 μm or 0.25 μm × 0.25 μm) on magnetic tapes as well as on polaroid film. The data were then played back to a T.V. monitor for mass analysis via a computer program (13). The mass measurement was accomplished by determining the amount of electron scattering per individual protein molecule minus a background (carbon film) obtained by averaging electron scattering from bare foil nearby the molecule. The actual molecular weights were determined using TMV for calibration. DNA contour lengths were measured by a Numonics graphics calculator using a Wild dissecting microscope to view the film.

RESULTS AND DISCUSSION

In the electron micrographs obtained from the STEM, the DNA molecules are qualitatively similar in appearance. The DNA molecules used in this work are released from the plasmids pXls11 by digestion with restriction endonuclease Hind III and purified by electrophoresis on agarose gel. The sequence of this somatic 5S DNA has been determined (9) and the sequence length is 882 base-pairs. The histogram of the measured length of DNA molecules are shown in Fig. 1. The measured lengths are converted to base-pairs (bp) assuming the factor 3.00 bp/nm appropriate to form B DNA (14). As the measured length is consistent with the sequence length, this implies that the measured length of the DNAs are compatible with form B DNA. The mean length of the DNA is 855 bp with a standard deviation of 36 bp which is found to be shorter than the sequence length. One explanation for the short measurement may be that a typical DNA contains one or more loops or folds which are not resolved and which for objectivity must be measured straight through. When TFIIIA is mixed with Xls 5S DNA, complexes of TFIIIA and DNA can be visualized in the STEM. TFIIIA interacts specifically with an intragenic control region in the 5S RNA genes of *Xenopus* (2, 3, 4). The positions of the TFIIIA binding specifically to the Xls 5S DNA were measured using a numonics graphics calculator and the mass determined using the STEM. With this technique the number of the electrons scattered to an annular detector

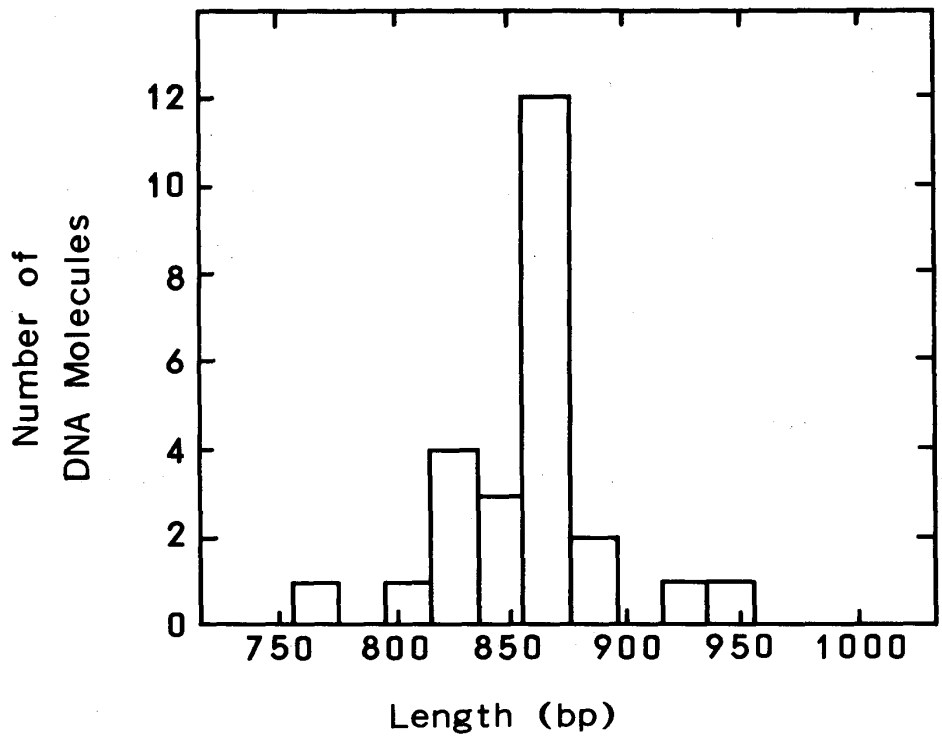

Fig. 1. Histogram of measured contour lengths of the *X. laevis* somatic 5S DNA molecules. The measured lengths were converted to base-pairs assuming the factor 3.00 bp/nm appropriate to form B DNA.

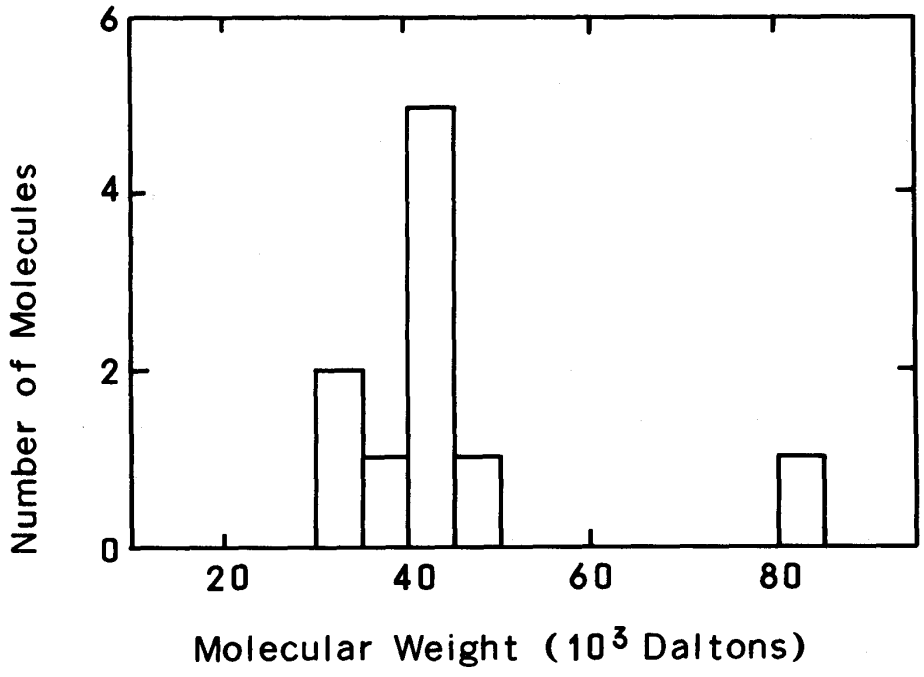

Fig. 2. Histogram of molecular weights of TFIIIA. Molecular weights were determined by integration of electron scattering intensities over each molecule.

for each of the 512 × 512 picture elements in a scan are recorded on magnetic disk and the STEM image can then be studied by recall from the disk. For pictures of interest, the disk is copied to tape for further analysis using a separate computer system (13). The scattered electron number is proportional to the mass contained within the element (including the mass of the supporting foil) (11). Thus, a number proportional to the net mass of a molecule is obtained by subtracting the counts from an equal number of picture elements of bare foil nearby. The actual molecular weight of a molecule is determined by calibration using the accurately known mass per unit length of TMV. TFIIIA which specifically bound to Xls 5S DNA were found to have molecular weights in the range of 34,000 to 80,000. A histogram of the molecular weights of TFIIIA measured using the STEM is shown in Fig. 2. The mean molecular weights excluding the 80,000 object was 40 ± 4 kilodaltons (kD) (n=9), which is quite similar to the values obtained by gel migration (38—40 kD) (10, 15). Fig. 3 shows a monomer of TFIIIA specifically bound to DNA observed in the STEM. One can see a molecule at twice this value, or 80 kD, which presumably represents a dimer of TFIIIA (Fig. 4). Some higher order aggregates not bound to DNA can also be seen. The observed error of 10% is consistent with the well-established STEM error analysis (6, 16) and is due to statistical fluctuation in the number of scattered electrons plus variation in the thickness of the supporting substrate which limits the accuracy of background subtraction. The mass analysis is particularly valuable in that it allows one to interpret whether apparently different morphologies none the less represent proteins with the same mass.

Measurement of the binding of TFIIIA to Xls 5S DNA revealed that approximately 36% of the DNA were found to have TFIIIA bound specifically. A TFIIIA molecule of monomer mass (42 kD) binding at the position 180 bp to 201 bp from the end of DNA is shown in Fig. 5. This binding position corresponds to the 5S RNA gene residue between 78 and 95 which includes the essential contact points of the internal control region investigated by Sakonju *et al.* (5). TFIIIA with a dimer mass (80 kD) was found to bind at 174 bp to 213 bp from the downstream end of the DNA which contacts almost of the 3′ end of the control region (Fig. 4).

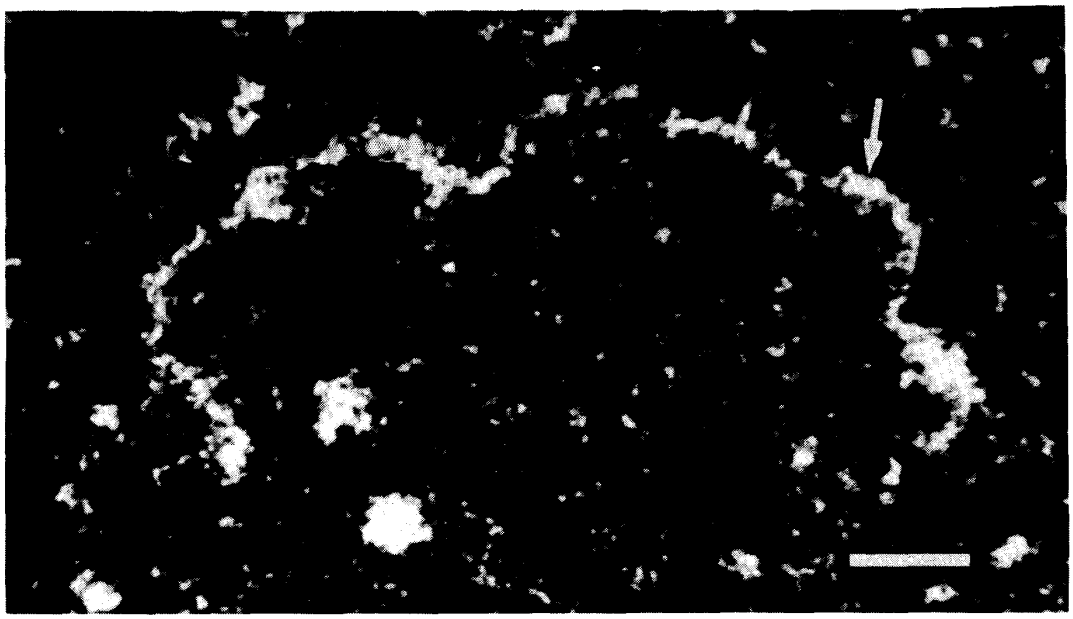

Fig. 3. STEM micrograph of TFIIIA (arrow) bound specifically to Xls DNA. The preparation is unstained and unshadowed. The bar represents 20 nm.

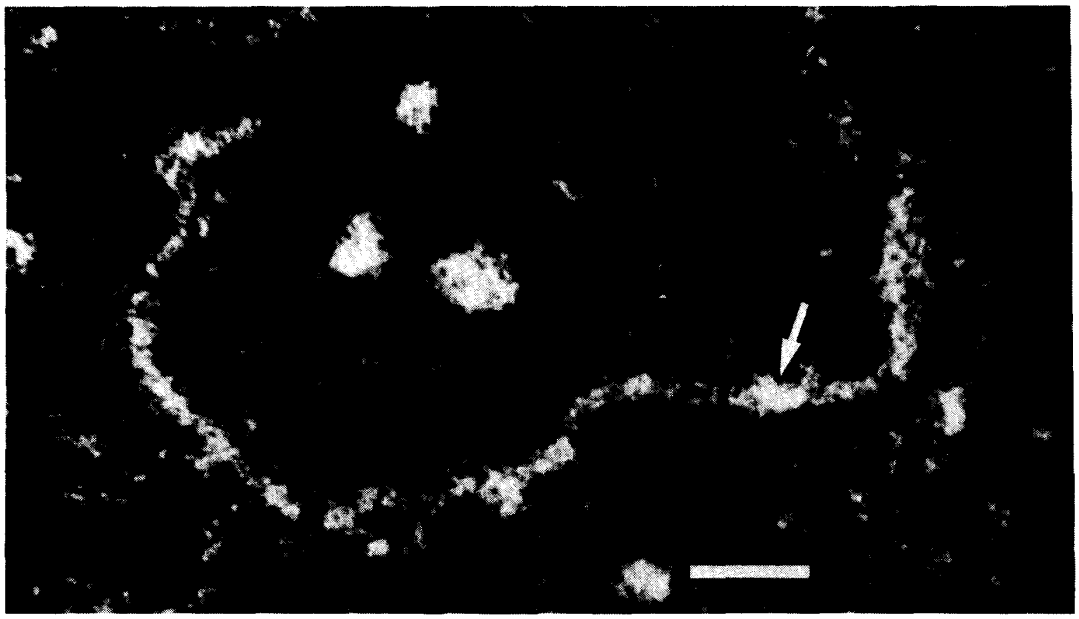

Fig. 4. STEM micrograph of a TFIIIA dimer (arrow) bound specifically to Xls DNA. The bar represents 20 nm.

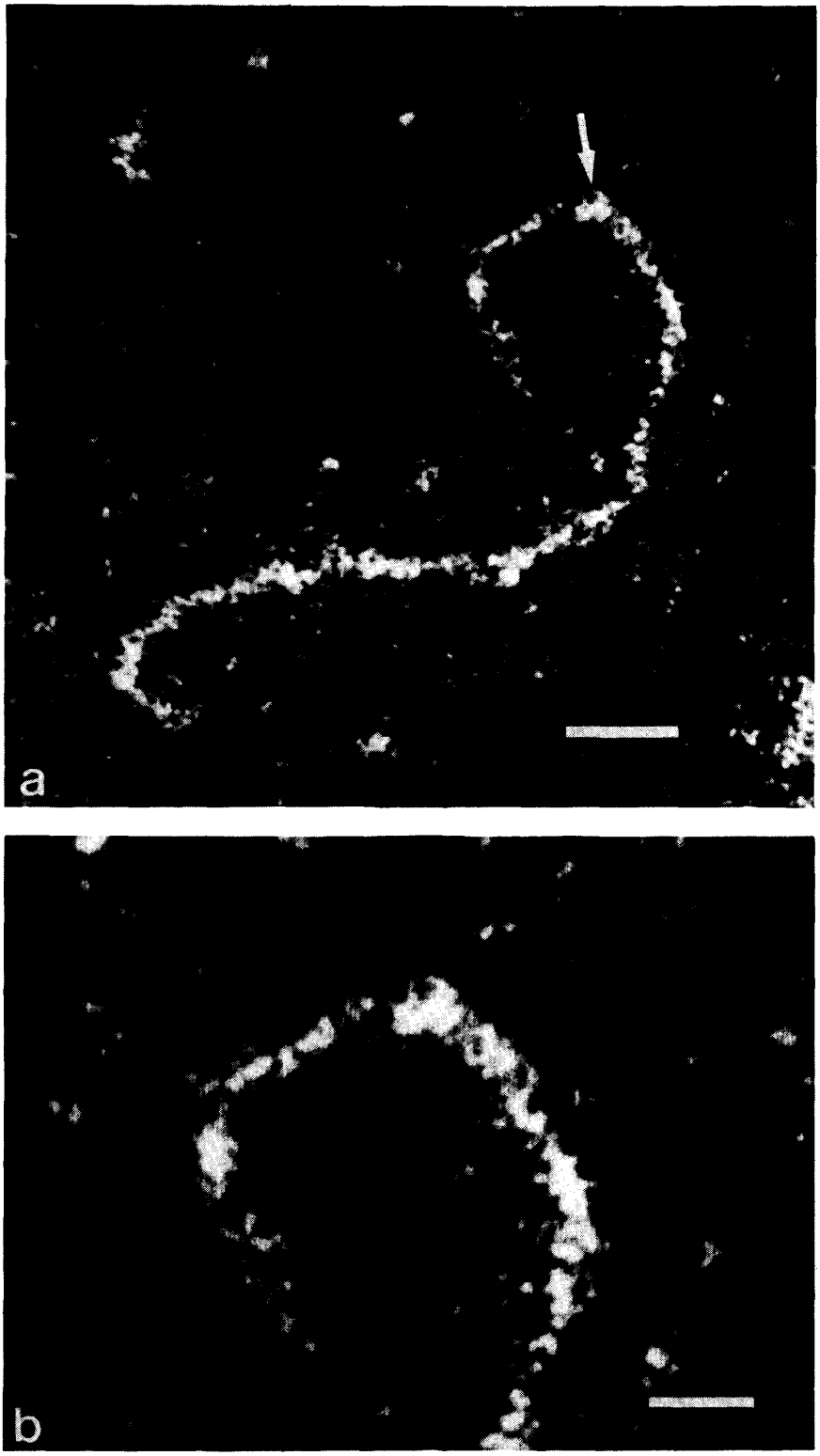

Fig. 5. (a) STEM micrograph of TFIIIA (arrow) bound to the internal control region of Xls DNA. The bar represents 20 nm. (b) High-magnification view of the TFIIIA of (a). The bar represents 10 nm.

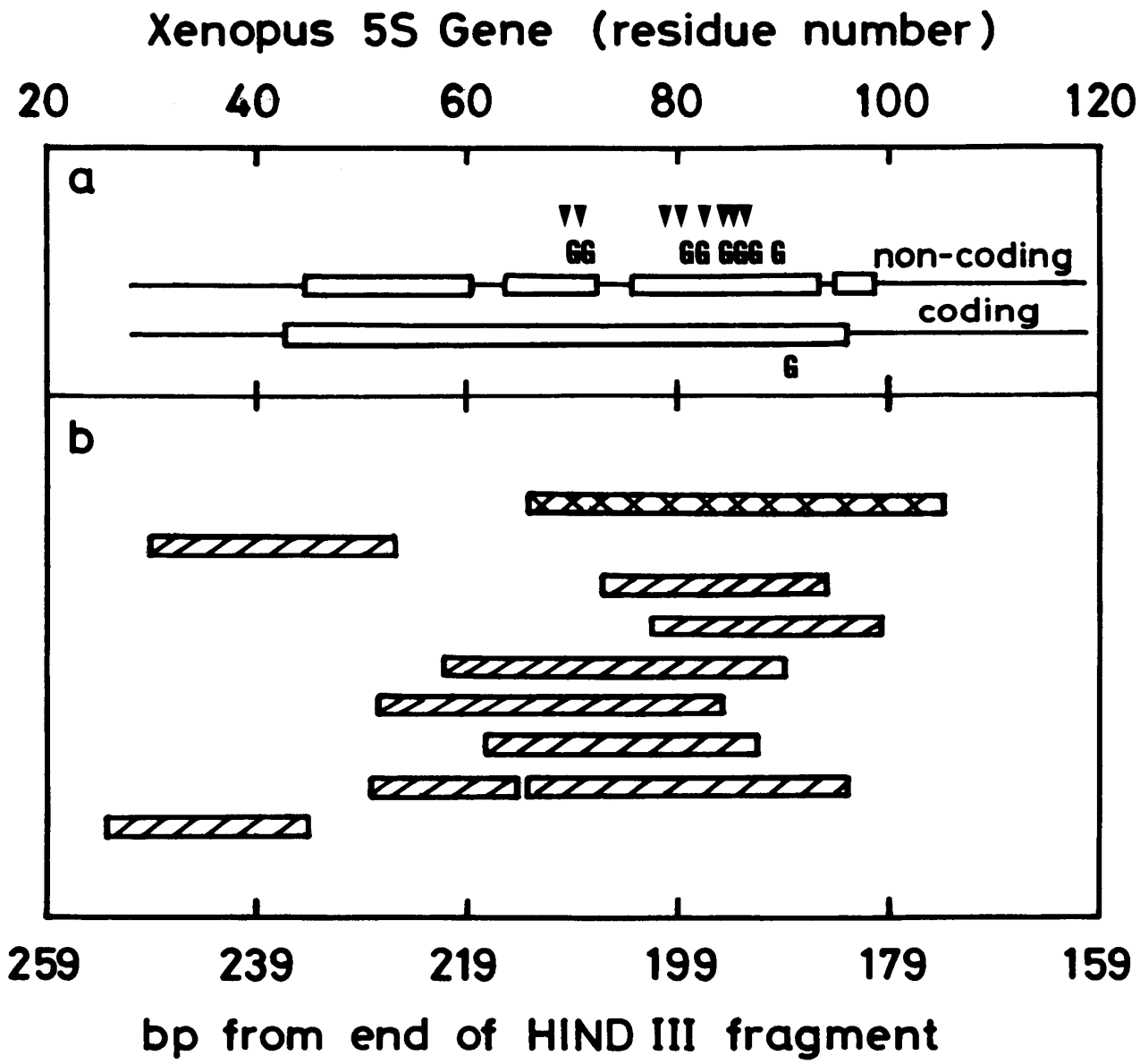

Fig. 6. Measurement of the binding of TFIIIA to *X. laevis* somatic 5S DNA. Positions on the 5S DNA are indicated by residue number of the gene on the top and by base-pairs from the end of the HIND III fragment at the bottom. (a) Positions protected from DNAase I digestion by TFIIIA are indicated by open box. Guanine residues that interfere, when methylated, with binding of the TFIIIA are indicated by letters G and phosphate residues that make contact with the TFIIIA are marked by solid triangles. The data are from Sakonju, S. and Brown, D. D., 1982. (b) Observed binding of TFIIIA to Xls DNA are indicated by hatched boxes (monomer mass) and cross hatched box (dimer mass).

Fig. 6 shows the observed binding of TFIIIA to Xls DNA and the corresponding contact points with the internal control region studied by Sakonju and Brown (5). For a stoichiometry of 8 : 1 molar ratio of TFIIIA to DNA, 80% or more of the specifically bound particles are monomers whereas at 60 : 1 molar ratio approximately the reverse ratio of binding is found (Simon and Hough (1983), unpublished results). From Fig. 6 it may be suggested that TFIIIA has a tendency to bind at the 3′ end of the control region. This result is consistent with the strong contacts to residues 70 and 71 and also to residues between 81 and 91 as found by Sakonju *et al.* (5). Some TFIIIA binding at or beyond the upstream end of the control region is found, and with no protein binding at the downstream site. It is not clear yet whether these represent specific or non-specific binding. It is clearly important to establish conditions which will distinguish between specific and non-specific binding. One experiment which is planned is to carry out an infinite dilution by passing the reaction mixture over a suitable sizing column. It is known that the protein-DNA complexes will run ahead of unbound protein and therefore the complexes have been effectively infinitely diluted. Under such circumstances, non-specifically bound proteins are expected to dissociate much faster than those specifically bound.

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